

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A method for quantifying the expression of target gene sequences of interest in a sample, comprising the steps of: (i) amplifying ninety-five to one-thousand and thirteen cDNA molecules derived from a sample by polymerase chain reaction in the presence of ~~a plurality of~~ ninety-five to one-thousand and thirteen amplification primer sets suitable for amplifying target gene sequence of interest, and in the presence of ninety-five to one-thousand and thirteen oligonucleotide probes complementary to a region of an amplified target gene sequence, said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest, and each of which is disposed between the ninety-five to one-thousand and thirteen primer sets amplifying a given target gene sequence of interest, and (ii) quantifying at least one of the target gene sequences amplified in step (i) in a real-time PCR in which the product of step (i) is divided into a plurality of aliquots and said real-time PCR quantifying in step (ii) is performed on said aliquots,

wherein the amplifying of step (i) comprises ninety-five to one-thousand and thirteen PCR primer pairs, wherein each primer of the ninety-five to one-thousand and thirteen PCR primer pairs in step (i) is present at a concentration of 30-45 picomolar, wherein the quantifying in step (ii) comprises PCR amplifying with at least one of the primer pairs in step (i) and at least one of the oligonucleotide probes in step (i).

2. (Original) The method of claim 1 in which the amplification of step (i) is further carried out in the presence of a reverse transcriptase such that the polymerase

chain reaction is reverse-transcription polymerase chain reaction and wherein the one or more cDNA molecules is obtained from mRNA derived from the sample.

3. (Original) The method of claim 1 in which the one or more cDNA molecules comprise a cDNA library.
4. (Canceled)
5. (Original) The method of claim 1 in which the polymerase chain reaction of step (i) is carried out for a number of cycles such that the amplification remains in the linear range.
6. (Original) The method of claim 1 in which the amplification in step (i) is achieved with a thermostable DNA polymerase.
7. (Canceled)
8. (Previously presented) The method of claim 1 in which the label is a fluorophore.
9. (Previously presented) The method of claim 1 in which said at least one oligonucleotide probe is selected from the group consisting of 5'-exonuclease probes, stem-loop beacon probes and stemless beacon probes.
10. (Withdrawn). The method of claim 1 in which said at least one oligonucleotide probe comprises a plurality of oligonucleotide probes, each of which is complementary to a region of a different amplified target gene sequence of interest.
11. (Withdrawn) The method of claim 10 in which the product of step (i) is divided into a plurality of aliquots and said quantifying in step (ii) is performed on said aliquots.

12. (Withdrawn) The method of claim 11 wherein the number of aliquots is equal to the number of primer pairs used in the multiplex amplification.

13. (Withdrawn) The method of claim 12 in which step (ii) comprises amplifying the product in each aliquot by polymerase chain reaction in the presence of an amplification primer set suitable for amplifying one of the target sequences of the plurality.

14. (Withdrawn) The method of claim 13 in which the amplifying in step (ii) is further carried out in the presence of an oligonucleotide probe complementary to a region of a different amplified target gene sequence of interest, wherein each probe in step (ii) comprises one of the oligonucleotide probes in step (i).

15. (Withdrawn) The method of claim 12 in which the sequences of the amplification primer sets of step (i) are the same as the sequences of the amplification primer sets of step (ii).

16. (Withdrawn) The method of claim 11 in which the amplifying in step (ii) is further carried out in the presence of a molecule that produces a detectable signal when bound to a double-stranded polynucleotide suitable for monitoring the amplification reaction as a function of time.

17. (Withdrawn) The method of claim 16 in which the molecule is selected from the group consisting of an intercalating dye and a minor groove binding dye.

18. (Withdrawn) The method of claim 17 in which the molecule is selected from the group consisting of SYBR® green I and ethidium bromide.

19 - 21. (Canceled)

22. (Previously presented). The method of claim 1 in which an observed efficiency of amplification is greater than 70%

23. (Previously presented) The method of claim 1 in which an observed efficiency of amplification is greater than 90%.

24 - 42. (Canceled)

43. (Previously presented) The method of claim 1 in which the amplification is carried out in the presence of uracil N-glycosylase.

44. (Previously presented) The method of claim 1 in which the amplifying the ninety-five to one-thousand and thirteen cDNA molecules comprises as many as fourteen PCR cycles.